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L2: Entry 1 of 1

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Nov 9, 1999

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TITLE: Methods and compositions for regulated transcription and expression of heterologous genes

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

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US-CL-CURRENT: 800/287; 435/183, 435/415, 435/416, 435/419, 435/468, 435/469, 435/69.1, 435/70.1, 536/23.6, 536/24.1, 536/24.5, 800/286, 800/294, 800/298, 800/306, 800/312, 800/314, 800/320, 800/322

### CLAIMS:

#### What is claimed is:

- 1. A DNA construct comprising as operably linked components in the direction of transcription, a promoter region obtainable from a gene selected from the group consisting of a napin gene, an EA9 gene and an acyl carrier protein gene; a DNA sequence of interest other than the native coding sequence of said gene; and a transcription termination region, wherein said components are functional in a plant cell, and wherein said DNA construct is flanked by T-DNA.
- 2. The DNA construct according to claim 1, wherein said DNA sequence of interest encodes an enzyme.
- 3. The DNA construct according to claim 1, wherein said DNA sequence of interest is an antisense sequence.
- 4. A plant cell having an altered phenotype as a result of expression of a DNA construct according to claim 1.
- 5. The plant cell according to claim 4, wherein said DNA construct is flanked by T-DNA.
- 6. The plant cell according to claim 5 wherein said plant cell is one from the group consisting of a soybean cell and rapeseed cell.
- 7. The plant cell according to claim 4, wherein said DNA sequence of interest encodes an enzyme.
- 8. The plant cell according to claim 4, wherein said DNA sequence of interest

- is an antisense sequence.
- 9. A plant comprising cells comprising a DNA construct according to any one of claims  $1,\ 2$  or 3.
- 10. The plant according to claim 9, wherein said plant is dicotyledonous.
- 11. Seed obtained from a plant according to claim 10.
- 12. Seed having a DNA construct according to claim 1.
- 13. The seed according to claim 12, wherein said seed is an oil seed or a grain seed.
- 14. The seed according to claim 12, wherein said seed is from a dicotyledonous plant.
- 15. The seed according to claim 14, wherein said seed is from a plant of the genus Brassica.
- 16. The seed according to claim 14, wherein said dicotyledonous plant is selected from the group consisting of cotton, soybean, safflower and sunflower.

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L1: Entry 1 of 1

File: USPT

Aug 28, 2001

US-PAT-NO: 6281410

DOCUMENT-IDENTIFIER: US 6281410 B1

TITLE: Methods and compositions for regulated transcription and expression of heterologous genes

DATE-ISSUED: August 28, 2001

INVENTOR - INFORMATION:

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US-CL-CURRENT: 800/287; 435/468, 435/469, 800/286, 800/294, 800/306, 800/312, 800/317.4, 800/322

#### CLAIMS:

What is claimed is:

1. A method for obtaining a plant which produces at least one seed having a modified phenotype, said method comprising:

transforming a host plant cell with a DNA construct under genomic integration conditions, wherein said construct comprises as operably linked components in the direction of transcription, a promoter region obtainable from a gene of a Brassica plant, wherein transcription of said gene is preferentially regulated in plant seed tissue, a DNA sequence of interest other than the native coding sequence of said gene, and a transcription termination region, wherein said components are functional in a plant cell,

whereby said DNA construct becomes integrated into a genome of said plant cell,

regenerating a plant from said transformed plant cell, and

growing said plant under conditions whereby said DNA sequence of interest is expressed and a seed having said modified phenotype is obtained.

2. A method of altering the phenotype of plant seed tissue as distinct from other plant tissue, said method comprising:

growing a plant which produces at least one seed, wherein said plant comprises cells containing a DNA construct integrated into their genome, said DNA construct comprising, in the 5' to 3' direction of transcription, a transcriptional initiation region from a gene of a Brassica plant, wherein transcription of said gene is preferentially regulated in a plant seed tissue, a DNA sequence of interest other than the coding sequence native to said

transcriptional initiation region, and a transcriptional termination region, whereby transcription of said DNA sequence of interest is controlled by said transcriptional initiation region and plant seed tissue having an altered phenotype is obtained.

- 3. The method according to claim 1 or 2, wherein said DNA construct is flanked by T-DNA.
- 4. The method according to claim 3, wherein said plant is selected from the group consisting of soybean, rapeseed and tomato.
- 5. The method according to claim 1 or 2 wherein said DNA sequence of interest encodes an enzyme.
- 6. The method according to claim 1 or 2 wherein said DNA sequence of interest is an antisense sequence.
- 7. The method according to claim 1 or 2 wherein said gene is transcribed during seed maturation.
- 8. The method according to claim 7 wherein said gene is transcribed from about day 11 to day 30 postanthesis.
- 9. The method according to claim 1 or claim 2, wherein said DNA sequence of interest is a structural gene.
- 10. The method according to claim 1 or claim 2, wherein said DNA sequence of interest is an open reading frame encoding an amino acid sequence.
- 11. The method according to claim 1 or claim 2, wherein said transcriptional initiation region further comprises a translational initiation region.
- 12. A method according to claim 1, wherein said promoter region is from a gene encoding a storage protein.
- 13. The method according to claim 2, wherein said transcriptional initiation region further comprises a translational initiation region and said DNA sequence of interest is an open reading frame encoding an amino acid sequence.
- 14. A method according to claim 2, wherein said transcriptional initiation region is from a gene encoding a storage protein.
- 15. A method for obtaining a plant which produces at least one seed having a modified phenotype, said method comprising:

transforming a host plant cell with a DNA construct under genomic integration conditions, wherein said construct comprises as operably linked components in the direction of transcription, a promoter region obtainable from a gene of a Brassica plant, wherein said gene is preferentially transcribed during seed embryogenesis, a DNA sequence of interest other than the native coding sequence of said gene, and a transcription termination region, wherein said components are functional in a plant cell,

whereby said DNA construct becomes integrated into a genome of said plant cell,

regenerating a plant from said transformed plant cell, and

growing said plant under conditions whereby said DNA sequence of interest is expressed and a plant having said seed with a modified phenotype is obtained.

16. A method of altering the phenotype of plant seed tissue as distinct from other plant tissue, said method comprising:

growing a plant, wherein said plant comprises cells containing a DNA construct integrated into their genome, said DNA construct comprising, in the 5' to 3' direction of transcription, a transcriptional initiation region from a gene of a Brassica plant, wherein said gene is preferentially transcribed during seed embryogenesis, a DNA sequence of interest other than the coding sequence native to said transcriptional initiation region, and a transcriptional termination region, whereby transcription of said DNA sequence of interest is controlled by said transcriptional initiation region and plant seed tissue having an altered phenotype is obtained.

- 17. The method according to claim 15 or 16, wherein said gene is transcribed from about day 7 to day 40 postanthesis.
- 18. A method for modifying a genotype of a plant to impart a desired characteristic to seed as distinct from other plant tissue, said method comprising:

transforming under genomic integration conditions, a host plant cell with a DNA construct comprising in the 5' to 3' direction of transcription, a transcriptional initiation region from a gene of a Brassica plant, wherein transcription of said gene is preferentially regulated in a plant seed tissue, a DNA sequence of interest other than the native coding sequence of said gene, and a transcriptional termination region, whereby said DNA construct becomes integrated into the genome of said plant cell;

regenerating a plant from said transformed host cell; and

growing said plant to produce seed under conditions whereby said DNA sequence of interest is expressed and a plant having a modified genotype is obtained.

- 19. The method according to claim 18, wherein said DNA construct is flanked by T-DNA.
- 20. The method according to claim 18, wherein said plant is a Brassica plant.
- 21. The method according to claim 18, wherein said DNA sequence of interest encodes an enzyme.
- 22. The method according to claim 18, wherein said DNA sequence of interest is an antisense sequence.
- 23. The method according to claim 18, wherein said plant is a soybean or a tomato plant.
- 24. A method for modifying transcription in seed tissue as distinct from other plant tissue, said method comprising:

growing a plant capable of developing seed tissue under conditions to produce seed, wherein said plant comprises cells containing a DNA construct integrated into their genome, said DNA construct comprising, in the 5' to 3' direction of transcription, a seed-specific transcriptional initiation region obtained from a gene of a Brassica plant, a DNA sequence of interest other than the coding sequence native to said transcriptional initiation region, and a transcriptional termination region, whereby transcription of said DNA sequence of interest is controlled by said seed-specific transcriptional initiation region.

- 25. The method according to claim 24, wherein said DNA sequence of interest is an antisense sequence.
- 26. The method according to claim 24, wherein said plant is of the genus Brassica.

- 27. The method according to claim 24, wherein said transcriptional initiation region further comprises a translational initiation region and said DNA sequence of interest is an open reading frame encoding an amino acid sequence.
- 28. The method according to claim 24, wherein said plant is a soybean or a tomato plant.
- 29. A method to selectively express a heterologous DNA sequence of interest in seed tissue as distinct from other plant tissue, said method comprising:

growing a plant capable of developing a seed tissue under conditions to produce seed, wherein said plant comprises cells having a genomically integrated DNA construct comprising, as operably linked components in the 5' to 3' direction of transcription, a seed-specific transcriptional initiation region and a translational initiation region each obtained from a gene of a Brassica plant, a DNA sequence of interest other than the coding sequence native to said transcriptional initiation region, and a transcriptional termination region downstream of said DNA sequence of interest, whereby said DNA sequence of interest is expressed under control of said seed-specific transcriptional and translational initiation region.

- 30. The method according to claim 29, wherein said plant is of the genus Brassica.
- 31. The method according to claim 29, wherein said plant is a soybean or a tomato plant.
- 32. A method for modifying transcription in plant seed tissue as distinct from other plant tissue, said method comprising:

growing a plant wherein said plant comprises cells containing a DNA construct integrated into their genome, said construct comprising:

- a transcriptional initiation region from a gene of a Brassica plant wherein said gene is preferentially expressed in plant seed tissue, a DNA sequence of interest other than the coding sequence native to said transcriptional initiation region, wherein said DNA sequence of interest is controlled by said transcriptional initiation region, whereby transcription in said plant seed tissue is modified as distinct from other plant tissue.
- 33. The method according to claim 32, wherein said DNA construct further comprises a translational initiation region and a termination region.
- 34. A method to selectively express a heterologous DNA sequence of interest in plant seed tissue as distinct from other plant tissue, said method comprising: growing a plant wherein said plant comprises cells containing a DNA construct integrated into their genome, said construct comprising as operably linked components in the direction of transcription, a promoter region from a gene of a Brassica plant that is preferentially expressed in plant seed tissue and a DNA sequence of interest, wherein said DNA sequence of interest is other than the coding sequence native to said promoter region and is expressed under the control of said promoter region whereby said DNA sequence of interest is expressed in plant seed tissue.
- 35. A method for obtaining a plant which produces at least one seed having a modified phenotype, said method comprising:

transforming a host plant cell with a DNA construct under genomic integration conditions, wherein said construct comprises as operably linked components in the direction of transcription, a promoter region obtainable from a gene, wherein transcription of said gene is preferentially regulated in plant seed tissue and said gene is selected from the group consisting of a napin gene, an

ACP gene, and an EA9 gene, a DNA sequence of interest other than the native coding sequence of said gene, and a transcription termination region, wherein said components are functional in a plant cell,

whereby said DNA construct becomes integrated into a genome of said plant cell,

regenerating a plant from said transformed plant cell, and

growing said plant under conditions whereby said DNA sequence of interest is expressed and a plant having said seed with a modified phenotype is obtained.

36. A method of altering the phenotype of plant seed tissue as distinct from other plant tissue, said method comprising:

growing a plant, wherein said plant comprises cells containing a DNA construct integrated into their genome, said DNA construct comprising, in the 5' to 3' direction of transcription, a transcriptional initiation region from a gene, wherein transcription of said gene is preferentially regulated in plant seed tissue and said gene is selected from the group consisting of a napin gene, an ACP gene and an EA9 gene, a DNA sequence of interest other than the coding sequence native to said transcriptional initiation region, and a transcriptional termination region, whereby transcription of said DNA sequence of interest is controlled by said transcriptional initiation region and plant seed tissue having an altered phenotype is obtained.

- 37. The method according to claim 35 or 36, wherein said plant is a dicotyledonous plant.
- 38. The method according to claim 37, wherein said dicotyledonous plant is selected from the group consisting of rapeseed, soybean, safflower, sunflower and tomato.
- 39. The method according to claim 35 or 36, wherein said DNA construct further comprises a translational initiation region.
- 40. The method according to claim 35 or 36, wherein said DNA construct is flanked by T-DNA.
- 41. The method according to claim 35 or 36, wherein said DNA sequence of interest comprises an open reading frame encoding an amino acid sequence.
- 42. The method according to claim 35 or 36, wherein said DNA sequence of interest encodes an enzyme.
- 43. The method according to claim 35 or 36, wherein said DNA sequence of interest is complementary to an mRNA endogenous to a plant cell.
- 44. A method for obtaining a plant which produces at least one seed having a modified phenotype, said method comprising:

transforming a host plant cell with a DNA construct under genomic integration conditions, wherein said construct comprises as operably linked components in the direction of transcription, a promoter region obtainable from a gene of a Brassica plant encoding a seed storage protein, wherein transcription of said gene is preferentially regulated in plant seed tissue, a DNA sequence of interest other than the native coding sequence of said gene, and a transcription termination region, wherein said components are functional in a plant cell,

whereby said DNA construct becomes integrated into a genome of said plant cell,

regenerating a plant from said transformed plant cell, and

growing said plant under conditions whereby said DNA sequence of interest is expressed and a plant having said seed with a modified phenotype is obtained.

45. A method of altering the phenotype of plant seed tissue as distinct from other plant tissue, said method comprising:

growing a plant, wherein said plant comprises cells containing a DNA construct integrated into their genome, said DNA construct comprising, in the 5' to 3' direction of transcription, a transcriptional initiation region from a gene of a Brassica plant encoding a seed storage protein, wherein transcription of said gene is preferentially regulated in a plant seed tissue, a DNA sequence of interest other than the coding sequence native to said transcriptional initiation region, and a transcriptional termination region, whereby transcription of said DNA sequence of interest is controlled by said transcriptional initiation region and plant seed tissue having an altered phenotype is obtained.